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Fringe-free, Background-free, Collinear Third Harmonic Generation FROG Measurements for Multiphoton Microscopy

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Collinear pulse measurement tools useful at the full numerical aperture (NA) of multiphoton microscope objectives are a necessity for a quantitative characterization of the femtosecond pulses focused by these systems. In this letter, we demonstrate a simple new technique, for characterizing the pulse at the focus in a multiphoton microscope. This technique, a background-free, fringe-free, form of frequency-resolved optical gating, uses the third harmonic signal generated from a glass coverslip. Here it is used to characterize 100 fs pulses (typical values for a multiphoton microscope) at the focus of a 0.65 NA objective.

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Multiphoton microscopy is an important tool for studying biological structure and function. The image resolution and efficiency of multiphoton microscopy depends critically on the intensity in the sample produced at the focal spot of a high numerical (NA) aperture objective. Thus, it is highly desirable to characterize the pulse under the same conditions as used in the imaging process. Ideally the pulse measurement tool would also take advantage of signals and media inherent to the imaging process. In an earlier paper [1], we addressed these issues by demonstrating a new collinear, background free method for spatial-temporal pulse characterization at high numerical aperture that utilized third harmonic generation from a glass coverslip. This previous measurement technique was primarily limited to making simple autocorrelation measurements to characterize the pulse width because the appearance of fringes complicated the phase retrieval required for a frequency-resolved optical gating (FROG) measurement [2]. In the present work, we demonstrate for the first time, a third-order measurement technique to allow full pulse characterization in a multiphoton microscope at the focus of a high NA objective without forming interferometric fringes.

There is a well-developed collection of FROG techniques for characterization of ultra-fast laser pulses[2]. The different forms of FROG are distinguished by the nature of the nonlinearity and whether they can be performed collinearly. A second-order nonlinearity generally gives stronger signal (an important consideration for oscillator pulses), while a third-order nonlinearity eliminates the time-ambiguity inherent to the second-order processes. One such form, which has proven to be a standard tool for ultrashort pulses measurement, is second harmonic (SHG) FROG[3]. The technique is straightforward to implement, incorporating a nonlinear crystal as the medium in which

the second order effect is generated. In previous work, Fittinghoff et al used type II phase matching at the focus of a high-NA objective to avoid background and fringing in a collinear geometry[4, 5]. This method proved quite effective, and was used to characterize 20-fs pulses at the focus of a 1.2 NA objective. The main limitation of this technique is the requirement of a type II nonlinear crystal – it's simply not a tool found in the biological laboratory. More recently, Amat-Roldan et al [6] employed starch as the source of second-order nonlinearity. The material is thin and inexpensive, and proved capable of generating interferometric SHG measurements under the imaging conditions found in a microscope. In this case, prior to retrieval, the fringes are removed computationally.

Third harmonic generation (THG) FROG in a noncollinear geometry was first demonstrated by Tsang et al [7]. It is advantageous in that it only requires a simple glass cover slip to provide an interface through which the third harmonic is generated (a THG signal is typically present at any dielectric interface.) It is insensitive to the choice of laser wavelength, is not subject to phase-matching and group velocity walk off limitations, and lacks the time ambiguity of SHG FROG [3]. Moreover, the optical arrangement of the THG FROG allows the characterization of both spatial profiles at the focus of a high NA objective through a 3^{rd}-order transverse autocorrelation.

Collinear, background-free THG sum-mixing signals can be obtained by using two input pulses with opposite circular polarization. There is no THG from each individual beam due to angular momentum conservation; however, third-order mixing of combined *R*- and *L*-circular polarization states is allowed. There are therefore two possibilities for the polarization state of the TH signal: left and right. Suppose the delayed

and reference signals are in the *L*- and *R*-circular states, respectively. If one photon from the delayed beam is used, the THG signal will be produced in an *L* state given by

$$E_{sig}^{L}(t,\tau) = E^2(t)E(t-\tau) \tag{1}$$

However, if two photons from the delayed beam are used, the signal will be in the *R* state, and

$$E_{sig}^{R}(t,\tau) = E(t)E^2(t-\tau) \tag{2}$$

Note that since the time delay appears on opposite orders, and both signals are produced simultaneously, the sum of these signals will have a time symmetry ambiguity. However, since the signals are orthogonal polarization states, they will not interfere provided the detector is insensitive to polarization. The THG autocorrelation measurements presented in [1] were insensitive to polarization, and were therefore fringe-free. In using a spectrometer to spectrally resolve the signal, the grating will select a component of each signal and interference fringes will occur. The signal will still possess the direction of time ambiguity and is computationally too complex for routine characterization.

In the present work, we circumvent this difficulty by using a circular polarizer to select only one of the above signals. This yields a fringe-free, background-free FROG trace without the time ambiguity found in THG autocorrelations. Depending on the beam selected by the circular polarizer, the resulting signal is written as

$$I_{FROG}^{THG}(\omega,\tau) = \left| \int_{-\infty}^{\infty} E_{sig}^{L,R}(t,\tau) e^{-i\omega t} dt \right|^2 \tag{3}$$

denoting the intensity of the left OR right circular third harmonic beam. The form of the signal here is the same as for non-collinear THG FROG. Thus a standard non-collinear THG FROG algorithm can be used to perform the phase retrieval.

For this experiment we use a home-built, neodymium phosphate glass laser that produces approximately 100 fs pulses centered at 1064 nm. The average power incident on the sample is 10 mW per beam. The fundamental beam is sent through a half-wave plate and into a polarization sensitive Michelson interferometer (see Fig. 1). The beam is split 50/50 between the two arms, where the delay in one arm is computer controlled. The beams are recombined in the second beam splitter, and follow a co-linear path into the microscope system. A quarter wave plate (centered at 1064 nm) is used to obtain two opposite circularly polarized beams at the fundamental frequency. Zeiss Achroplan 40x, 0.65 NA objectives are used for both the focusing and collection objectives. The fundamental is focused on the back surface of a simple glass coverslip to produce third harmonic light, which is collected and collimated by the second objective. The THG signal is sent through a quarter wave plate (centered at 355 nm), with the axis orientated to obtain two orthogonal linearly polarized beams. A polarizer then selects the optimum polarization state for the spectrometer where a 16 cm tube lens focuses the THG light onto the spectrometer slit. A KG3 filter is used to block the fundamental light from entering the spectrometer. We note that the chromatic aberration in the relay optics also helps to reduce the power of the fundamental entering the spectrometer.

The alignment of the quarter-wave plate and polarizer to eliminate the interference fringes is critical. First the polarizer is aligned to coincide with the direction of maximum spectrometer throughput. The quarter-wave plate is then adjusted during

scanning at high resolution (0.25fs/step) to eliminate fringes in the signal. Figure 2 shows the FROG signal over a short scan range with fringes. Without fringes in the signal, it is now possible to scan at a much coarser time-step. The FROG trace with the waveplate properly adjusted is shown in figure 3. Retrieval algorithms (Femtosoft Technologies) give a temporal pulse FWHM of 107 fs and a spectral FWHM bandwidth of 12 nm with a minimum FROG error of 0.0037. The final grid size used was 64x64. The measured and retrieved FROG traces are shown in figure 3 for comparison, the extracted intensity and phase is shown in figure 4. The traces are virtually identical indicating excellent agreement. Notably acquisition rates were spectrometer limited. The acquisition per time step was 1 second (64 seconds total) limited by the rate at which the spectrometer could be reliably shuttered.

In conclusion, we have demonstrated fringe-free, background-free THG FROG for application in high NA imaging systems for the first time. It allows pulse characterization at the focus of the full NA of the optical system using a nonlinear media readily available in microscopes – the glass coverslip. This is not only convenient, it is achromatic – this technique can be employed over the complete tuning range of Ti:sapphire laser systems, as well as with lasers operating in the 1 μm range and beyond. Notably this new FROG technique has important implications for high-dynamic range pulse characterization. It is completely background-free, and thus can be used to investigate the wings of laser pulses produced in high-intensity laser systems.

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List of Figure Captions

Fig. 1 Experimental layout for background-free, collinear, THG measurement

Fig. 2 Partial FROG scan, showing the fringing that is inherently present in the trace without the addition of the circular analyzer.

Fig. 3 Upper figure – measured THG FROG trace, Lower figure – retrieved THG FROG trace. In each trace, the vertical dimension is wavelength (.25 nm per step), and the horizontal dimension is time (13.3 fs per step).

Fig. 4 Retrieved intensity (arb. Units) and phase (radians).

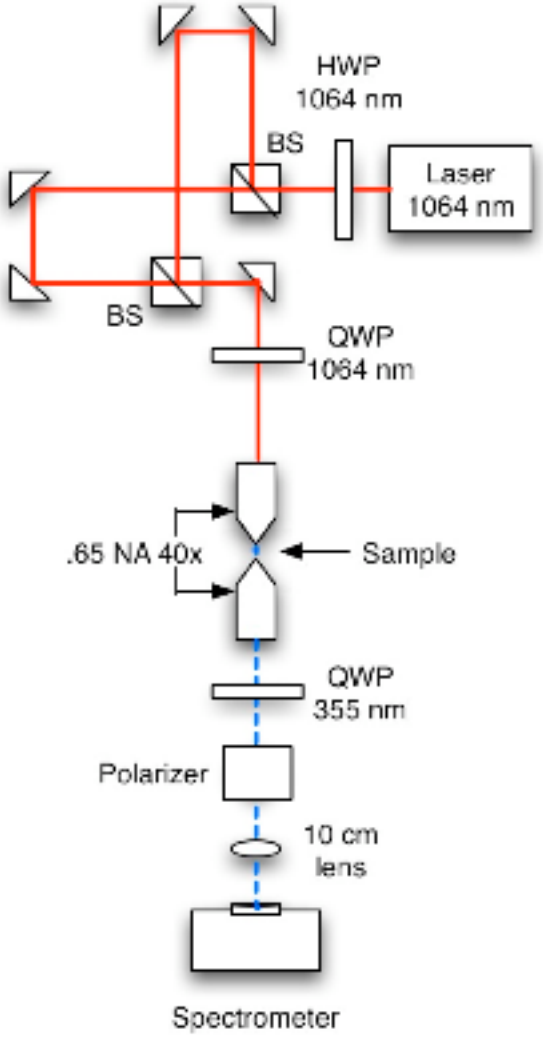

HWP
1064 nm
BS
Laser
1064 nm
BS
QWP
1064 nm
.65 NA 40x
Sample
QWP
355 nm
Polarizer
10 cm
lens
Spectrometer

Figure 1

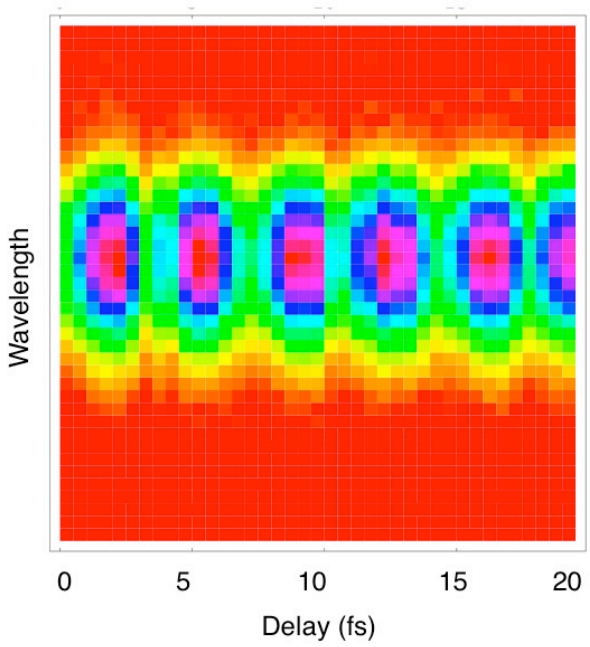
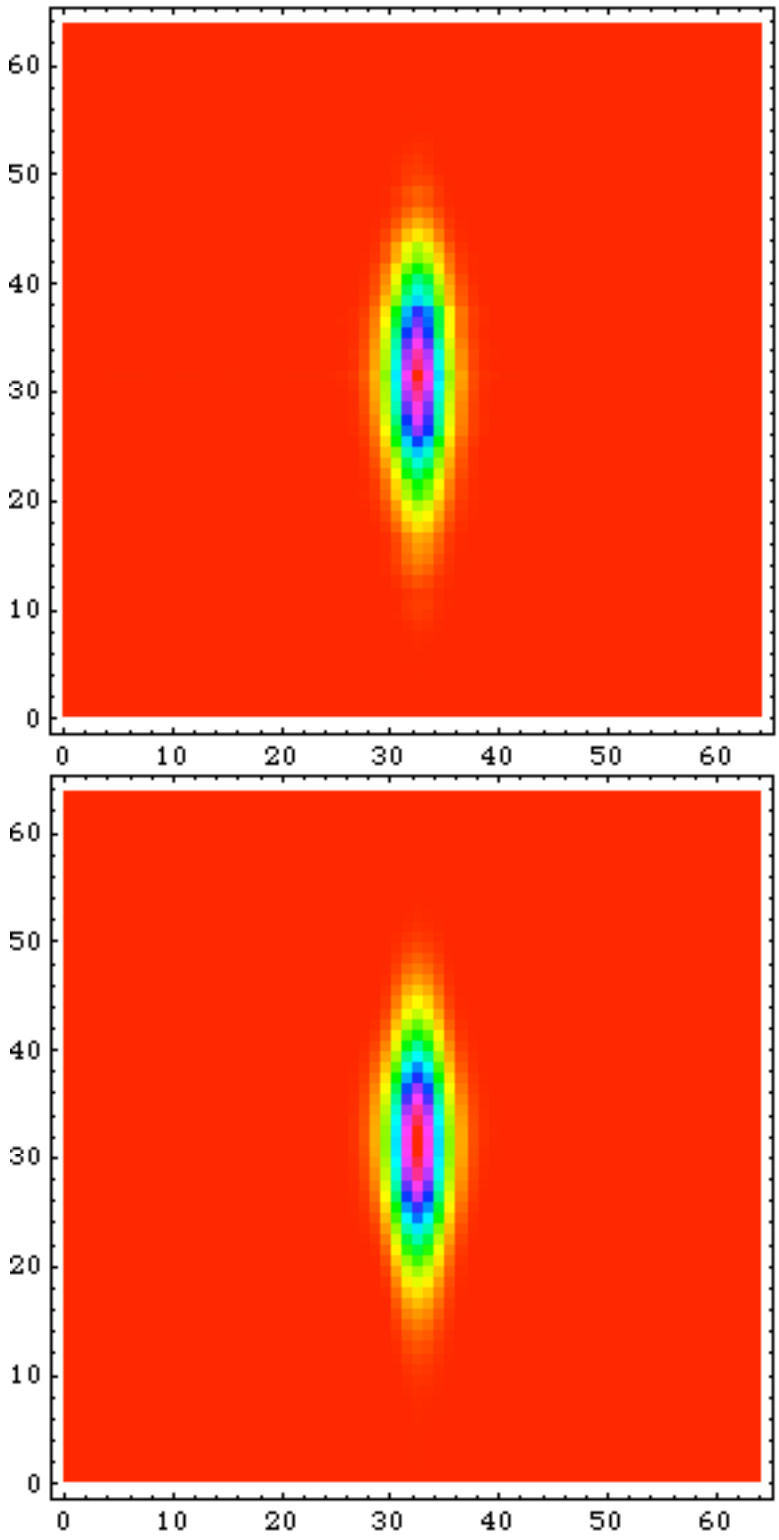


Figure 2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50
60

Figure 3

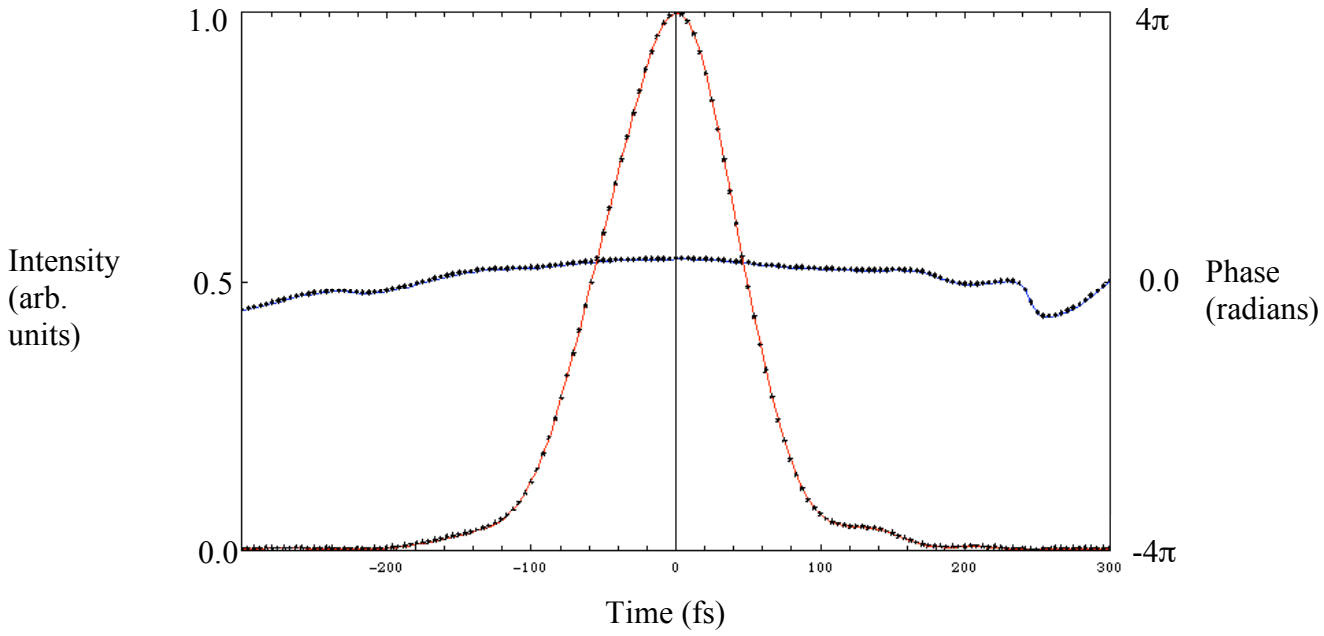
Intensity (arb. units)
1.0
0.5
0.0
4π
0.0
-4π
Phase (radians)
-200
-100
0
100
200
300
Time (fs)

Figure 4

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